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Memorandum

JUL 29 1992

Date

From Chief, Hematology/Pathology Branch, Division of Clinical Laboratory Devices, Office of Device Evaluation, Center for Devices and Radiological Health

Subject Draft Guidance Document for 510(k) Submission of Fecal Occult Blood Tests

To Interested Parties

We have developed a draft document entitled, "Review Criteria for the Qualitative Assessment of Fecal Occult Blood In Vitro Diagnostic Devices" that includes a generic model package insert for guaiac fecal occult blood tests for professional use. Since these documents concern devices we will be reviewing, it is intended to assist manufacturers in the preparation of marketing submissions for these types of devices.

We are soliciting your ideas, recommendations, and comments regarding the enclosed draft guidance document and model package insert. We will appreciate receiving your comments so we can incorporate as many improvements as possible in a revision. Additional copies of these documents may be obtained through the Office of Small Manufacturers Assistance from Geoffrey Clark, (301) 443-6597.

Please address comments to:

Willard D. Stewart
Chief, Hematology/Pathology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and Radiological
Health
Food and Drug Administration
1390 Piccard Drive
Rockville, MD 20850

Willard D. Stewart

Attachment

REVIEW CRITERIA FOR THE QUALITATIVE ASSESSMENT OF FECAL OCCULT BLOOD
IN VITRO DIAGNOSTIC DEVICES

This is a flexible document representing the current major concerns and suggestions regarding in vitro diagnostic fecal occult blood devices. It is based on (1) current basic science, (2) clinical experience, (3) previous submissions by manufacturers to the FDA, and (4) the Safe Medical Devices Act of 1990 (SMDA) and (5) FDA regulations in the Code of Federal Regulations (CFR). As advances are made in science and medicine and changes in implementation of Congressional legislation, these review criteria will be re-evaluated and revised as necessary.

PURPOSE OF THE GUIDANCE DRAFT

This document is an adjunct to the 21 CFR Parts 800-1299. While it is not to supersede the CFR, this document provides additional guidance and clarification on what information is necessary before the Food and Drug Administration (FDA) can clear a device for marketing.

DEFINITION

This document discusses all generic types of devices intended for use in clinical laboratories, doctors' offices and/or over-the-counter use as an in vitro diagnostic test for the qualitative measurement of fecal occult blood.

PRODUCT CODE: KHE-81

REGULATION NUMBER: 21 CFR § 864.6550

CLASSIFICATION: Class II

PANEL: Hematology

REVIEW REQUIRED: 510(k)

I. CLINICAL INDICATIONS/SIGNIFICANCE/INTENDED USE OF THE DEVICE

It has been reported that false positive guaiac results occur from a diet of red or rare meats due to the pseudoperoxidase activity of myoglobin or blood present in the tissues. While restriction of intake of raw fruits and vegetables containing peroxidases, e.g., cantaloupe, horseradish, radish, turnip, and broccoli¹, is recommended by the American Cancer Society, its value has not been established. A single positive result should be investigated, even in the absence of dietary restrictions.²

Certain drugs, such as aspirin, and non-steroidal anti-inflammatory drugs may increase gastrointestinal bleeding and cause false positive test results. Iron compounds also have been reported to cause false positive reactions. Ascorbic acid (vitamin C) taken at levels greater than 250 mg/day can cause false negative guaiac test results. Ascorbic acid should be avoided for two days before and during testing.² Drugs should be avoided for seven days before and during the testing period.³

Intermittent tumor bleeding and irregular distribution of blood in the feces also contribute to false negative results. For these reasons, it has been recommended that two areas from each of three consecutive stools be sampled, but the appropriateness of this recommendation remains untested.⁴

Rehydration of stored guaiac slides does increase test sensitivity, however, at the expense of many false positive results. The American Cancer Society recommends that slides should not be rehydrated. They also recommend that the

delay between sample collection and testing should not exceed 6 days. Others recommend 4 days as the time limit.⁵

Hemoglobin tends to be altered chemically and to lose its pseudoperoxidase activity as it passes through the gastrointestinal tract. Upper gastrointestinal tract bleeding is therefore thought to be less likely to produce a positive test result than is lower gastrointestinal tract bleeding.²

Theoretically immunological fecal occult blood tests may also be more sensitive to lower than upper gastrointestinal bleeding because of increased degradation of globin vs. the heme that is detected by the guaiac slide tests.² Whether or not they are more specific for colorectal cancer vs. upper gastrointestinal problems needs to be established for the 510(k) submission.

If the test employs one or more monoclonal antibodies, the fact that it will react positively with all genetically abnormal hemoglobins should be established. The site of amino acid substitution for hemoglobins S and C appears to be very immunogenic. One product did not react positively with blood from persons with sickle cell disease.

Significance of False Negative and Positive Test Results.

Patients who have asymptomatic cancer or adenoma or other conditions and negative test results, could potentially be given false reassurance and delay reporting symptoms. Such a risk is difficult to quantify, and could potentially be ameliorated by proper counseling regarding the significance of a negative test result.² Patients with significant risk factors, such as family history of colorectal cancer, should be screened regularly following guidelines of the American Cancer Society.

II. DEVICE DESCRIPTION:

Identification. An occult blood test is a device used to detect occult blood in urine or feces. (Occult blood is blood present in such small quantities that it can be detected only by chemical tests of suspected material, or by microscopic or spectroscopic examination.)

Provide a concise discussion to include the following as appropriate: (1) a brief historical summary of all test methodologies used to detect fecal occult blood; (2) merits/advantages and limitations/disadvantages of this device methodology(ies) compared to other available methodologies; (3) all specimen types/matrix(ices) used by this test methodology(ies). Matrix is defined as the milieu containing the analyte in the patient sample submitted for analysis (hereinafter "specimen type").⁶

III. CLINICAL AND NONCLINICAL LABORATORY STUDIES: SPECIFIC PERFORMANCE CHARACTERISTICS

FDA requests different types and amounts of data and statistical analyses in applications to market in-vitro diagnostic devices. The amount and type of data requested depends on: 1) the test analyte, 2) the intended use (which determines whether the application is a 510(k), or if an original Premarket Approval application (PMA), 3) whether the test is quantitative or qualitative, and 4) whether the data design is independent or paired.

Establish the performance of the device by comparison to a another legally marketed guaiac slide fecal occult blood test. Prove all claims for performance and substantial equivalence.

A. Analytical/Laboratory/In Vitro Studies

The FDA recommends submission of the following data to establish the substantial equivalence of fecal occult blood tests:

1. Antibody Sensitivity/Specificity/Cross-Reactivity/Interference Studies

For monoclonal antibody tests only, demonstrate that this test will be positive with whole blood samples from persons with hemoglobinopathies more prevalent in the United States, e.g., sickle cell disease [Hemoglobin (Hb) SS], Hb CC, etc. Contraindicate testing patients with hemoglobinopathies not tested in the limitations section of the package insert.

For immunological tests including both monoclonal and polyclonal antibody-based tests, demonstrate that there is no test interference caused by the following immunologically related substances: hemoglobins from beef, chicken, fish, horse, pig, rabbit, goat, sheep; myoglobin or ground up meat extracts prepared from beef, chicken, fish, horse, pork or ham, rabbit, goat, sheep.

If some type of tested raw meat extract gives a false positive result, either the user can be warned not to eat that type of meat before or during the testing period, or the manufacturer may undertake further testing. Next test cooked meat. If it is negative, the package insert should have clear instructions that well-cooked X meat only should be eaten. Rare meat may give a false positive result. If cooked meat gives a false positive result, the manufacturer may want to determine if it still gives a false positive result after a trip through the intestinal tract by testing persons who have eaten X meat, etc.

Test all immunologically-based tests and in-the-bowl tests as appropriate for the following potentially interfering foods using the assay system: peroxidase containing raw vegetables and fruits commonly contraindicated in guaiac test package inserts, e.g., horseradish, red radish, raw turnip, cauliflower, broccoli, parsnip, cantaloupe, etc.⁷

Test all "in-the-bowl" and immunologically-based tests (if the sample is taken from the toilet water) for the following potential interferents in toilet water: toilet bowl refreshers, bluing agents cleaners and deodorizers which may be present in the toilet bowl water, fluoride, chloride, iron, and other metals. Alternatively, control procedures may be employed in the step-by-step procedure to control for false positive results.

Test all immunologically-based and in-the-bowl tests for potentially interfering drugs⁷ that might be commonly used by patients tested for fecal occult blood, e.g. vitamin C (ascorbic acid), therapeutic iron preparations, etc.

Alternatively, add a statement to the Limitations Section of the Package Insert that the device has not been tested for cross-reactivity or interference of one or more of the above substances.

2. Reproducibility and Repeatability Studies⁸

Include reproducibility studies performed in laboratories, physician's offices, and for over-the-counter use as claimed.

For qualitative tests, perform a combined reproducibility and in vitro limits of detection study for all new tests. This may be accomplished by repeating increasing and defined dilutions of normal human hemoglobin in water or stool. The dilutions should be sufficiently closely spaced around the positive-negative cutoff range to adequately determine the reproducibility around the test positive-negative test cutoff.

For example, dilute hemoglobin in distilled water to the following concentrations: 1mg/mL, 2mg/mL, 4mg/mL, and 6mg/mL (equivalent to 0.1, 0.2, 0.4, and 0.6 grams hemoglobin per 100 grams of occult-blood-negative stool, respectively, and approximately 1, 2, 4, and 6 mL of blood per 100 grams of negative stool). Use these dilutions to test the sensitivity of the test and compare the reactions of this test to those of at least one other legally marketed guaiac slide test. Repeat each dilution a sufficient number of times for an adequate reproducibility study.

3. Comparison of In Vitro Limits of Detection.

For all new tests except "Float in the-bowl" tests, compare the in vitro limits of detection of the new device to those of another legally marketed guaiac slide fecal occult blood test. This may be accomplished by using increasing dilutions of normal human hemoglobin in water or stool. The dilutions should be sufficiently closely spaced especially around the positive-negative cutoff range to adequately determine that the limits of detection are comparable. It is suggested that this study be combined with the reproducibility study described above. Report results in the Performance Characteristics section of the package insert.

4. Prozone Effect Studies (Immunological Tests Only)

Test a sample with a very high concentration of occult hemoglobin. (It is unnecessary to test a visibly bloody specimen). If the test result is positive, declare in the Performance Characteristics section of the package insert the highest concentration of hemoglobin tested that gives a positive result. This will be the quantitative level below which no interference from prozone effect was observed.

5. Specimen Collection and Handling

For immunologically based tests, provide data to demonstrate that weakly positive stool samples will give stable results under conditions simulating transport at 95°F. If no data is provided or data demonstrates that the sample is not stable under those conditions, instructions for transport of samples on ice to the Doctors office or laboratory should be given in the patient instruction sheet.

6. Positive and Negative Performance Monitors

Positive and negative performance monitors must be incorporated into the test design of all over-the-counter fecal occult blood tests. Provide data to demonstrate that positive and negative performance monitors of all tests which have them will correctly predict when the test is performing acceptably and when it is not. Subject the test to conditions which will inactivate it, e.g., U.V. light, excessive heat or cold, etc. Then demonstrate that the positive performance monitor will correctly monitor whether the test activity is or is not destroyed with a weakly positive sample of hemoglobin in water or stool.

7. Accuracy of Interpretation of Test Results by Lay Reader

For over-the-counter tests only, demonstrate that lay users can read the test results as accurately as professionals. Allow at least 50 non-professional lay persons over the age of 40 with various educational backgrounds to interpret the results of a variety of test specimens, including borderline positive samples. Duplicate specimens should be interpreted by personnel experienced in evaluating the type of device being tested and the results compared to those of the lay reader.

8. Data Required to Demonstrate Understandability of Product Labeling for Over-the-counter tests.

Once the home-use IVD product instructions are written, demonstrate that product labeling (instructions for use) are understandable by at least 50 non-professional lay persons over the age of 40 with varied educational backgrounds. Without any explanation from the manufacturer, have each person use the test following only the package insert directions. Have each individual provide the results of their testing to the manufacturer. Have a health care professional familiar with the test procedure evaluate the specimen to see if the specimen was adequately collected. Have each individual fill out a questionnaire to evaluate how well he/she understood the information provided in the package insert, the test directions, etc.

B. Clinical Investigations

In certain instances it may be necessary to require comparative clinical data to establish substantial equivalence, e.g., a new or unfamiliar methodology or technological feature is introduced in a device category in which clinical performance is claimed to be equivalent to another legally marketed device using "conventional" technology. Clinical data (e.g., from testing of actual clinical specimens in diagnosed subjects) may be required.

Describe all protocols for clinical studies. Plan the sample size that will be statistically sufficient to determine substantial equivalence. FDA requires only the minimum data necessary to arrive at a determination of substantial equivalence. Explain if clinically significant samples will be difficult to obtain, e.g., rare disease, no gold standard method, few non-exposed persons because of widespread prevalence of patients with disease exposure early in life, etc. It is recommended that testing should be performed at at least one outside testing facility.

The FDA requests submission of the following data to establish the substantial equivalence of fecal occult blood tests:

For immunological and in-the-bowl test, generate data to demonstrate that results obtained with the new immunological or "Float-in-the-bowl" test are substantially equivalent to those obtained with another legally marketed guaiac slide fecal occult blood test, i.e., have an acceptable number of false negative and false positive results. Use fecal specimens from a minimum of 50 patients which yield positive results and from a minimum of 100 asymptomatic persons over the age of 40. Investigate and attempt to resolve all discrepant results. Include positive test samples from patients that cover a wide range of gastrointestinal bleeding disorders, but especially conditions with low levels of bleeding and colorectal cancers which are still in curable stages. Perform side-by-side testing on samples which have been deposited into the toilet bowl or collected as instructed in the package insert.

IV. LABELING CONSIDERATIONS

See attached GENERIC MODEL PACKAGE INSERT FOR GUAIAEC FECAL OCCULT TESTS for examples.

Adequate directions for use for home-use in vitro diagnostic tests (IVD's) implies that the labeling should be simple, concise, easy to understand, make liberal use of illustrations and drawings, use bold print or other methods to highlight warnings and precautions, and provide color coding of reagent containers whenever practicable.

Intended Use

A typical intended use statement is: "ABC's *** test is a [TEST METHODOLOGY] test intended for the qualitative detection of fecal occult blood by laboratories or physicians offices. It is useful to detect blood found in a number of gastrointestinal disorders, e. g. diverticulitis, colitis, polyps, and colorectal cancer. This test is recommended for routine physical examinations, when hospital patients are first admitted, to monitor for bleeding in patients with iron deficiency anemia or recuperating from surgery, peptic ulcer, ulcerative colitis and other conditions, and in screening programs for gastrointestinal bleeding from any source."

Conditions for Use

Describe any special applications of the device or specific contraindications or indications for use not addressed in the Intended Use Statement, for example: "unmodified fecal occult blood tests are not recommended for use with gastric samples."¹⁰

Specimen Collection and Patient Preparation

Give information regarding sample type tested. For example, "the specimen is stool. It may be collected from the toilet bowl, from the toilet paper or caught in a clean cup. It is recommended that samples be collected from two different areas of each stool specimen."

Give instructions regarding how much sample is necessary, for example: "a thin smear should be applied to each of two guaiac windows."

State special precautions, for example: "all raw or red meat as well as high peroxidase-containing fruits and vegetables can cause false positive results."^{11,12} A red meat-free, high residue diet is recommended beginning two days prior to testing and continuing throughout the test period.^{11,12,13} Such a diet may help reduce the number of false positive results. It also provides roughage that may help uncover silent lesions which may bleed intermittently and may increase the rate of true positive reactions.^{1,14} The recommended diet will also increase the likelihood of a soft stool for greater ease in obtaining the sample. This diet should EXCLUDE red and rare meats, horseradish, raw fruits and vegetables like broccoli, cauliflower, red radish, cantaloupe, parsnips and turnips, or other high peroxidase containing vegetables, which can cause false positive results. An acceptable diet could include cooked fruit and vegetables such as spinach and corn as well as lettuce, prunes, grapes, and apples. Cereal, and well cooked fish and fowl are also acceptable.^{1,12}

"Since bleeding from gastrointestinal lesions may be intermittent, it has traditionally been recommended that specimens from three daily bowel movements be tested. However, it has been suggested that the test period be extended to test samples from six bowel movements. to compensate for fluctuations in bleeding and reduce false negative results, thus improving detection."¹⁵

State all interfering substances or conditions as follows. "A specimen should not be collected while patient presents bleeding hemorrhoids or is constipated, with cuts on hands or during or immediately after a menstrual period. Hands and test area should be kept clean and free from blood to avoid false positive reactions."

"Certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients."^{16,17} With the

physicians approval, such medications should be discontinued 7 days before and throughout the test period.¹⁶ Rectal medications should be discontinued."

"False negative results may be caused by ingestion of 250mg per day or more of ascorbic acid (Vitamin C).¹⁸ Such medications should be discontinued 2 days before and throughout the test period."

"The effect of therapeutic administration of various iron compounds on fecal occult blood tests has been the subject of some controversy. It has been reported that they may or may not produce false positive results with guaiac paper based tests.^{19,20,21} Test results should be interpreted with care, however, when patients receive therapeutic iron preparations."

Give instructions regarding stability of the specimens. State storage, handling or shipping instructions for the protection and maintenance of specimens, for example. "Guaiac slides prepared by the patient should be allowed to dry before development. No more than 4 to 6 days should elapse between preparation and testing. Patients should be instructed to return all slides to the physician or laboratory as soon as possible.^{5,15} The stored pad containing the sample should be protected from excessive heat, sunlight, fluorescent light, volatile chemicals, and humidity."

Quality Control²²

Include the following information in a separate "Quality Control" section. Give directions for interpretation of the results of performance monitors (satisfactory limits of performance). Conclude this "Quality Control" section with a statement similar to the following: "If performance monitors do not perform as expected, assay results are invalid."

Interpretation of Test Results

Explain how to interpret positive, negative, and equivocal/indeterminate/border-line results including their clinical significance. For example, "ANY TRACE of blue coloration is to be regarded as a positive for occult blood. An absence of blue indicates no detectable occult blood."

The conditions for reading results should be given, e.g., "These results should be read at room temperature (16-32°C) after 30 seconds and before 3 minutes of adding the developing reagent."

Give all any appropriate test limitations or interferences directly relating to interpretation of results, for example. "Neither the intensity nor the shade of blue from the positive Performance Monitor or any photographs provided should be used as a reference for the appearance of positive test results of samples."

State the clinical significance of results, for example. "Further testing and examinations should be performed by the physician to determine the exact cause and source of the occult blood in the stool. All patients who test positive regardless of diet should be followed up with additional diagnostic procedures.^{2,15}"

Limitations of the Procedure

List important test limitations and all known contraindications, with references, previously noted. Note also all interfering conditions mentioned under Specimen Collection and Handling Section.

"Results cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology."

"This test is to aid in diagnosis and is not intended to replace other diagnostic procedures such as proctosigmoidoscopic examination, full colonoscopy, barium enema, or other x-ray studies.²³"

"Because gastrointestinal lesions may bleed intermittently and blood in feces is not distributed uniformly, a negative result does not assure absence of lesions.^{4,24,25,26}"

"Guaiac slide tests should not be rehydrated before development because this gives excessive false positive results.²"

Expected Results

For example. "Guaiac impregnated paper has been extensively studied, and these clinical studies indicate that guaiac impregnated slide tests yield a positive result 3-5% of the time in screening programs. The percent of false positive results lies in the range of 1-2% under controlled conditions.^{11,27,28} Sensitivity (percent of subjects with the condition being sought who test positive) is difficult to estimate, but in series of patients with known colorectal cancer, 50-87% have been reported to yield positive reactions.^{25,29,30} Estimates of positive reactions with adenomatous bleeding have varied widely, and appear dependant to a degree on the size of polyp, with polyps less than 2 cms yielding less than 5% positive reactions.³¹"

V. BIBLIOGRAPHY

1. Macrae FA, St. John DJB, Caligiore P, Taylor LS, Legge JW. Optimal dietary conditions for Hemoccult testing. *Gastroenterology* 1982;82:899-903.
2. Knight KK, Fielding JE, Battista RN. Occult blood screening for colorectal cancer. *JAMA* 1989;261:587-93.
3. Grossman MI, Matsumoto KK, Lichter RJ. Fecal blood loss produced by oral and intravenous administration of various salicylates. *Gastroenterology* 1961;40:383-88.
4. Fleischer DE, Goldberg SB, Browning TH, Cooper JN, Friedman E, Goldner FH, Keefe EB, Smith, LE. Detection and surveillance of colorectal cancer. *JAMA* 1989;261:580-85.
5. Stroehlein JR, Fairbanks VF, Go VLW, et. al. Hemoccult stool tests: false-negative results due to storage of specimens. *Mayo Clin Proc* 1976;51:548-52.
6. Nomenclature and definitions for use in the national reference system for the clinical laboratory. 5(21):561. Order code NRSL8-P, ISBN 0273-3099
7. Information for authors. *Clin Chem* 1991;37:1-3.
8. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices - second edition; tentative guidelines. 1991:1-56. Order Code EP5-T2.
9. Clinical laboratory improvement amendments of 1988; final rule, 57 FR 7163, February 28, 1992.
10. Layne EA, et. al. Insensitivity of guaiac slide tests for detection of blood in gastric juice. *Ann Intern Med* 1981;94:774.

11. Ostrow JD, Mulvaney CA, Hansell JR, Rhodes RS. Sensitivity and reproducibility of chemical tests for fecal occult blood with an emphasis on false-positive reactions. *Am J Digest Dis* 1973;18:930-40.
12. Caligiore P, Macrae FA, St. John DJB, Rayner LJ, Legge JW. Peroxidase levels in food; relevance to colorectal cancer screening. *Am J Clin Nutr* 1982;35:1487-9.
13. Winawer SJ, Fleisher M, Baldwin M, Sherlock P. Current status of fecal occult blood testing in screening for colorectal cancer. *Ca* 1982;34:100-12.
14. Greigor DH. Detection of silent colon cancer in routine examination. *CA* 1969;19:330-7.
15. Gnauck R, Macrae FA, Fleisher M. How to perform the fecal occult blood test. *Ca* 1984;34:134-47.
16. Grossman MI, Matsumoto KK, Lichter RJ. Fecal blood loss produced by oral and intravenous administration of various salicylates. *Gastroenterology* 1961;40:383-88.
17. Doran J, Hardcastle JD. Bleeding patterns in colorectal cancer: the effect of aspirin and the implications for faecal occult blood testing. *Brit J Surg* 1982;69:711-3.
18. Jaffe RM, Kasten B, Young DS, MacLowry JD. False-negative stool occult blood tests caused by ingestion of ascorbic acid (vitamin C). *Ann Int Med* 1975;83:824-6.
19. Morgan TE, Roantree RJ. Evaluation of tests for occult blood in the feces. Significance of guaiac and orthotolidine tests after ingestion of iron. *JAMA* 1957;164:1664-67.
20. Lifton LJ, Kreiser J. False-positive stool occult blood tests caused by iron preparations. A controlled study and review of literature. *Gastroenterology* 1982;83:860-3.
21. Brayshaw JR, Harris F, McCurdy PR. The effect of oral iron therapy on the stool guaiac and orthotolidine reactions. *Ann Int Med* 1963;59:172-9.
22. National Committee for Clinical Laboratory Standards. Internal Quality Control Testing: Principles and Definitions; approved guideline. Villanova, PA. 1991. Order code C24-A:4.
23. Simon JB. Occult blood screening for colorectal carcinoma: a critical review," *Gastroenterology* 1985;88:820.
24. Griffith CDM, Turner DJ, Saunders JH. False negative results of Hemoccult test in colorectal cancer. *Br Med J* 1981;283:472.
25. Crowley ML, Freeman LD, Mottet MD, et.al. Sensitivity of guaiac- impregnated cards for the detection of colorectal neoplasia. *J Clin Gastroenterol* 1983;5:127-130.
26. Rosenfeld RE, Kochwa S, Kaczera Z, et al. Nonuniform distribution of occult blood in feces. *Am J Clin Pathol* 1979;71:204-9.
27. Hastings JB. Mass screening of colorectal cancer. *Amer J Surgery* 1974;127-288.

28. Greeger DH. A progress report - detection of colorectal cancer using guaiac slides. Cancer 1972; 22:360.

29. Macrea FD, St John DJB. Relationship between Hemocult sensitivity in patients with colorectal cancer or adenomas. Gastroenterology 1982;82:891-8.

30. Ribet A, Frexinos J, Escourrou J, et al. Occult Blood Tests and Colorectal Tumours. Lancet 1980;1:147.

31. Demers RY, Stawick LE, Demers, P. Relative sensitivity of fecal occult blood test and flexible sigmoidoscopy in detecting polyps. Prev Med 1985;14:55-62.

Version 7: 510K, June 22, 1992, Nina M. Chace

**GENERIC MODEL PACKAGE INSERT FOR GUAIAIC FECAL OCCULT BLOOD TESTS
FOR PROFESSIONAL USE**

INTENDED USE

The [MANUFACTURERS NAME] [TEST TRADE NAME] is a guaiac-based slide test intended for the qualitative detection of fecal occult blood in laboratories or physicians offices. It is a useful aid to detect bleeding caused by a number of gastrointestinal disorders, e. g., diverticulitis, colitis, polyps, and colorectal cancer. Fecal occult blood tests are recommended for use in 1) routine physical examinations 2) routine hospital testing 3) screening for colorectal cancer or gastrointestinal bleeding from any source.

Unbuffered fecal occult blood tests are not recommended for use with gastric samples.¹

SUMMARY and EXPLANATION

Van Deen is generally credited with the discovery that gum guaiac, a natural resin extracted from the wood of Guaiacum officinale, is useful in detecting occult blood² When adapted to the slide format, it became a widely used method for the detection of fecal occult blood, a sign of many gastrointestinal disorders. A positive result signals the necessity of follow-up by other diagnostic methods to determine the cause of the bleeding. The guaiac slide test overcomes the instability of guaiac solution and the hypersensitivity of benzidine and orthotolidine. This test is a simple, aesthetic, inexpensive test designed for use in the collection and preparation of stool specimens.

Guaiac slide test results cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology. False positive/negative reactions are known to be caused by a person's particular diet or medications (see Patient Preparation below). The test is intended as a preliminary screen and not as a replacement for other diagnostic procedures such as sigmoidoscopy, barium enema, and x-ray studies.

PRINCIPLE

The use of guaiac as a test for the presence of blood is based on the oxidation of colorless phenolic compounds present in guaiac to quinones, resulting in the production of a blue color.² If blood is present in the stool sample, the hematin portion of the hemoglobin molecule functions as a pseudoenzyme, catalyzing the release of oxygen from hydrogen peroxide which in turn causes the oxidation of guaiac. The test paper will turn blue in the presence of occult blood in the feces, but will remain uncolored in the absence of fecal occult blood. Quality control monitors on the guaiac slide indicate if the test is functioning correctly.

REAGENTS and MATERIALS SUPPLIED

Slides consisting of guaiac impregnated paper (100 provided). The positive performance monitor contains a substance impregnated into the paper which will turn blue if the product is functioning correctly. The negative performance monitor consists of guaiac-impregnated paper only.

Developing solution (30 mL). A mixture of 60-70% denatured ethyl alcohol and approximately 6% hydrogen peroxide.

Applicators

SUPPLIES NEEDED BUT NOT PROVIDED:

Timer to time 30 seconds +/- 2 seconds.

Post office approved mailing envelopes, 25 per pack (Cat. No. XXXX). Important Note: Current U.S. Postal Regulations prohibit mailing completed test slides in standard envelopes.

STORAGE and STABILITY

Reagents are stable when stored at controlled room temperature (XX-XX°C) (XX-XX°F) in the original package and protected from excessive heat or air flow, sunlight, U.V. irradiation, fluorescent light, humidity, volatile chemicals, (e.g. iodine or bleach). Do not refrigerate or freeze. Keep developer tightly capped to avoid evaporation. Do not use either component after the expiration date. The POSITIVE AND NEGATIVE MONITORS provide assurance that the slide and DEVELOPING SOLUTION are functioning according to product specifications.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use.

DEVELOPING SOLUTION IS AN IRRITANT. AVOID CONTACT WITH SKIN OR EYES. SHOULD CONTACT OCCUR, WASH IMMEDIATELY WITH WATER. DO NOT INGEST.

DEVELOPING SOLUTION IS FLAMMABLE. THE VIAL SHOULD BE PROTECTED FROM LIGHT, HEAT AND OPEN FLAME.

Patient specimens and all materials coming into contact with them should be handled as if capable of transmitting infection. Do not allow contact with the skin or mucous membranes. Dispose of test materials in an acceptable and safe manner. Never pipette by mouth

Do not substitute reagents from kits with different lot numbers or with components from other manufacturers.

PATIENT PREPARATION

Whenever possible, patients should follow a special diet for two days prior to and during the testing period. It should EXCLUDE red and rare meats, horseradish, raw fruits and vegetables like broccoli, cauliflower, red radish, cantaloupe, parsnips and turnips, or other high peroxidase containing vegetables, which can cause false positive results. An acceptable diet could include cooked fruit and vegetables such as spinach and corn as well as lettuce, prunes, grapes, and apples. Cereal, and well-cooked fish and fowl are also acceptable³. This diet will provide increased roughage. If any of the recommended foods are known to cause discomfort, patients should consult their physician.

Certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, corticosteroids and nonsteroidal anti-inflammatory drugs can cause gastrointestinal bleeding and thus give positive reactions. On the advice of the physician, these medications might be temporarily discontinued for 7 days prior to and during the test period.⁴

Dosages of greater than 250mg of vitamin C per day have been shown to cause false negative results⁵. The effect of therapeutic administration of various iron compounds on fecal occult blood tests has been the subject of some controversy. It has been reported that they may or may not produce false positive results with guaiac paper based tests^{6,7,8}. Test results should be interpreted with care when patients receive therapeutic iron preparations. Such medications should be discontinued 2 days before and throughout the test period.

Rectal medications should be discontinued.

SPECIMEN COLLECTION

A stool specimen may be collected from the toilet bowl, from the toilet paper or caught in a clean cup. It is recommended that smears be collected from two different areas of the stool from three consecutive bowel movements as closely spaced in time as possible^{9,10}. Samples from the outside of the stool will reflect conditions in the lower colon. Samples from the inside of the stool will be more representative of the upper gastrointestinal tract.

Since bleeding from gastrointestinal lesions may be intermittent, collect specimens from three different bowel movements.

INTERFERING CONDITIONS

Stool samples should not be collected if the patient is experiencing menstrual bleeding, constipation bleeding, bleeding hemorrhoids, cuts on hands or when rectal suppositories or medication is being used. These conditions could cause false positive results.

Hands and test area should be kept clean and free from blood to avoid false positive reactions.

SPECIMEN APPLICATION:

Application to the slide may be performed from a lubricated gloved finger (as after a rectal exam), or by use of the provided applicators. It is important that the stool specimen is applied as a very thin smear to each of the slide windows. Guaiac slides should be allowed to dry before development. No more than 4 to 6 days should elapse between preparation and testing. Patients should be instructed to return all slides to the physician or laboratory as soon as possible^{11,12}. Rehydration of the specimen is not necessary nor recommended.

SPECIMEN STORAGE

The stored pad containing the sample should be protected from excessive heat, sunlight, fluorescent light, volatile chemicals, and humidity.

METHOD

1. Supply all information requested on the front flap of the slide.
2. Open the front flap.
3. Using one of the applicators provided, collect a small stool specimen from the toilet. Apply a very thin smear in box A.
4. Reuse the applicator provided to obtain a second sample from a different part of the stool specimen. Apply a very thin smear inside box B. (On subsequent bowel movements, repeat above steps using additional slides.)
5. Allow the specimen to air dry, then close the cover.
6. Open perforated window on the back of the slide.
7. Apply two (2) drops of developer to the back side of boxes A and B.
8. Read results at room temperature (X - X°C) after 30 seconds and within two (2) minutes.

9. Record the results. Any trace of blue color, within or on the outer rim of the specimen, is positive for occult blood.
10. No trace of blue indicates no occult blood detected.

POSITIVE/NEGATIVE TEST PERFORMANCE MONITORS

Note: The procedure for developing the sample must be completed, interpreted and recorded before proceeding with the development of the test performance monitors.

1. To develop the monitors, place one or two drops of the developer between the positive and negative monitor boxes.
2. Read the results after 30 seconds and within two (2) minutes.
3. The positive monitor should turn blue, but the negative monitor should have no trace of blue.
4. If the performance monitors do not perform correctly, patient results are suspect. Contact the company at 1-(800)-XXX-XXXX.

STABILITY OF END PRODUCT

The color reaction is not permanent. Fading may occur after approximately 2 minutes.

QUALITY CONTROL

Positive and negative test performance monitors are provided on each slide. This specially treated area provides assurance that the guaiac-impregnated paper and the developer are reacting according to product specifications. The positive monitor should turn blue after 30 seconds and within two (2) minutes after the application of the developer, if the test system is reacting according to product specifications. The negative monitor should show no trace of blue color upon addition of developer. If the performance monitors do not perform correctly, patient results are suspect. Contact the company at 1-(800)-XXX-XXXX.

INTERPRETATION OF RESULTS

Results are to be read from the reverse side of the card. ANY trace of blue color within the specified time frame is positive for occult blood. An absence of blue indicates no detectable occult blood. These results should be read at room temperature (16-32°C) after 30 seconds and before 2 minutes of applying the developer solution. Within this time period, the proper functioning of the reagents is indicated by the positive monitor turning blue and the negative monitor remaining unchanged. Should the monitor reactions be different, the test results are invalid. Remember always to develop the test, interpret and record results before developing the performance monitors. Color blind persons should not interpret the results. Neither the intensity nor the shade of blue from the positive performance monitor should be used as a reference for the appearance of positive test results.

A positive result reflects the presence of occult blood in the stool. This does not mean the patient has tested positive for cancer or any other illness. False positive results may be caused by diet or medications. Further testing and examinations should be performed by the physician to determine the exact cause and source of the occult blood in the stool.

Because gastrointestinal lesions bleed intermittently and blood in feces is not distributed uniformly, all patients who test positive regardless of whether the

diet was followed strictly should be followed up with additional diagnostic procedures^{11,13}.

LIMITATIONS OF THE TEST

Results cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology. False negative results may occur because gastrointestinal lesions may bleed intermittently and blood in feces is not distributed uniformly¹⁴. False negative and positive reactions are known to be caused by a person's diet and medications (see PATIENT PREPARATION and INTERFERING CONDITIONS sections above). This test is to aid in diagnosis and is not intended to replace other diagnostic procedures such as sigmoidoscopy, barium enema, or other x-ray studies¹⁵.

Guaiac slide tests should not be rehydrated before development because this gives excessive false positive results¹³.

EXPECTED RESULTS

Guaiac impregnated paper has been extensively studied, and these clinical studies indicate that guaiac impregnated slide tests yield a positive result 3-5% of the time in screening programs. The percent of false positive results lies in the range of 1-2% under controlled conditions^{16,17,18}. Sensitivity (percent of subjects with the condition being sought who test positive) is difficult to estimate, but in series of patients with known colorectal cancer, 50-87% have been reported to yield positive reactions^{19,20,21}. Estimates of positive reactions with adenomatous bleeding have varied widely, and appear dependant to a degree on the size of polyp, with polyps less than 2 cms yielding less than 5% positive reactions²².

PERFORMANCE CHARACTERISTICS

Hemoglobin was diluted in distilled water to the following concentrations: 1mg/mL, 2mg/mL, 4mg/mL, and 6mg/mL (equivalent to 0.1, 0.2, 0.4, and 0.6 grams hemoglobin per 100 grams of stool, respectively, and approximately 1, 2, 4, and 6 mL of blood per 100 grams of stool). These dilutions were used to test the sensitivity of the test and compare the reactions of this test to those of several other commercially available guaiac slide tests. This test reacted positively at hemoglobin concentrations of 4mg/mL or greater in less than one (1) minute. At 2mg/mL this test gave a weak blue reaction in less than 2 minutes. Below 2mg/mL no positive reactions were observed. It was concluded that this test reacted positively to all hemoglobin levels above 2mg/mL, as did the several other commercially available guaiac slide tests tested.

REFERENCES:

1. Layne EA, et. al. Insensitivity of guaiac slide tests for detection of blood in gastric juice. *Ann Intern Med* 1981;94:774.
2. Irons GV Jr, Kirsner JB. Routine chemical tests for occult blood in the feces. *Am J of Med Sci.* 1965;249:247-60.
3. Caligiore P, Macrae FA, St. John DJB, Rayner LJ, Legge JW. Peroxidase levels in food; relevance to colorectal cancer screening. *Am J Clin Nutrit* 1982;35:1487-9.
4. Grossman MI, Matsumoto KK, Lichter RJ. Fecal blood loss produced by oral and intravenous administration of various salicylates. *Gastroenterology* 1961;40:383-88.

5. Jaffe RM, Kasten B, Young DS, MacLowry JD. False-negative stool occult blood tests caused by ingestion of ascorbic acid (vitamin C). *Ann Int Med* 1975;83:824-6.
6. Morgan TE, Roantree RJ. Evaluation of tests for occult blood in the feces. Significance of guaiac and orthotolidine tests after ingestion of iron. *JAMA* 1957;164:1664-67.
7. Lifton LJ, Kreiser J. False-positive stool occult blood tests caused by iron preparations. A controlled study and review of literature. *Gastroenterology* 1982;83:860-3.
8. Brayshaw JR, Harris F, McCurdy PR. The effect of oral iron therapy on the stool guaiac and orthotolidine reactions. *Ann Int Med* 1963;59:172-9.
9. Greigor DH. Detection of silent colon cancer in routine examinations. *Cancer* 1969;19:333-7.
10. Greigor DH. Occult blood testing for asymptomatic colon cancer. *Cancer* 1971;28:131-4.
11. Gnauk R, Macrae FA, Fleisher M. How to perform the fecal occult blood test. *Cancer* 1984;34:134-147.
12. Stroehlein JR, Fairbanks VF, Go VLW, et.al. Hemoccult stool tests: False-negative results due to storage of specimens. *Mayo Clin Proc* 1976;51:548-52.
13. Knight KK, Fielding JE, Battista RN. Occult blood screening for colorectal cancer. *JAMA* 1989;261:587-93.
14. Fleischer DE, Goldberg SB, Browning TH, Cooper JN, Friedman E, Goldner FH, Keefe EB, Smith, LE. Detection and surveillance of colorectal cancer. *JAMA* 1989;261:580-85.
15. Simon JB. Occult blood screening for colorectal carcinoma: a critical review, *Gastroenterology* 1985;88:820.
16. Ostrow JD, et.al. Sensitivity and reproducibility for fecal occult blood with an emphasis on false positive reactions. *Am J Dig Diseases* 1973;18:930.
17. Hastings JB. Mass screening of colorectal cancer. *Amer J Surgery* 1974;127-288.
18. Greigor DH. A progress report - detection of colorectal cancer using guaiac slides. *Cancer* 1972;22:360.
19. Crowley ML, Freeman LD, Mottet MD, et.al. Sensitivity of guaiac impregnated cards for the detection of colorectal neoplasia. *J Clin Gastroenterology* 1983;5:127-130.
20. Macrea FD, St John DJB. Relationship between Hemoccult sensitivity in patients with colorectal cancer or adenomas. *Gastroenterology* 1982;82:891-8.
21. Ribet A, Frexinos J, Escourrou J, et al. Occult Blood Tests and Colorectal Tumours. *Lancet* 1980;1:147.
22. Demers RY, Stawick LE, Demers P. Relative sensitivity of fecal occult blood test and flexible sigmoidoscopy in detecting polyps. *Prev Med* 1985;14:55-62.